# Mutation Detection Meets Flow Cytometry, with superRCA® Technology

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# Introduction to Rarity

## Technology

Based on decades of research, the innovative superRCA technology enables the detection of low-level nucleic acid sequences

## The Need

Highly sensitive and specific assays are needed for improved diagnostics and treatment monitoring

## Background

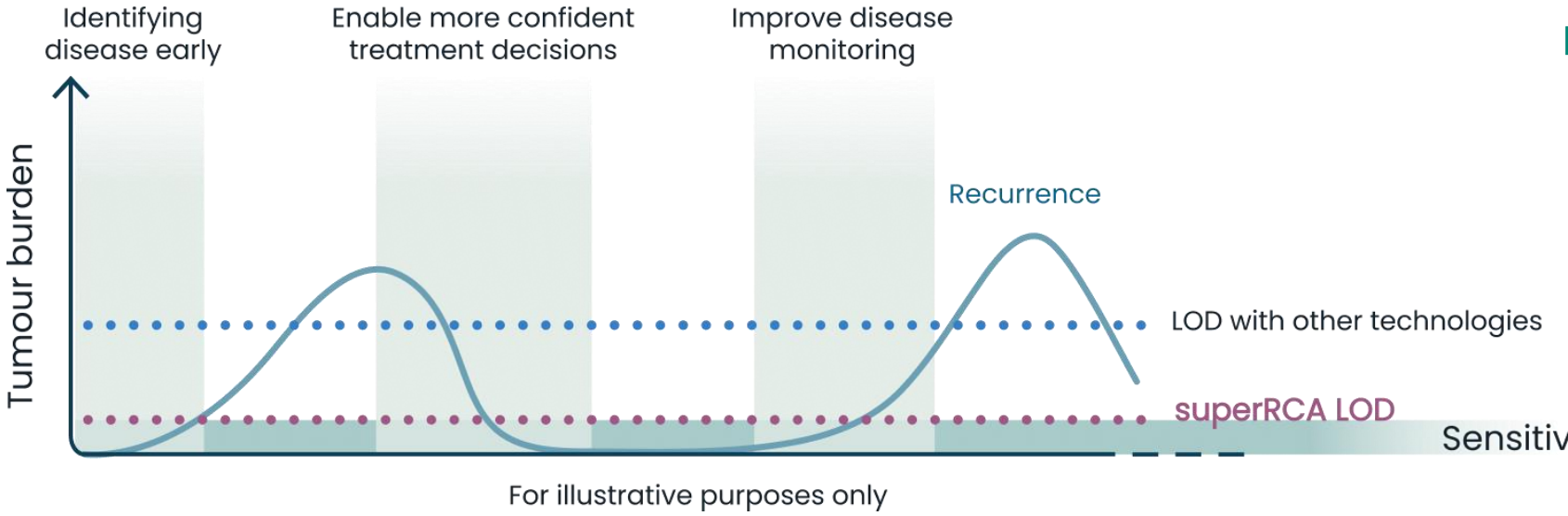
Rarity was founded in 2021, as a spin out from Uppsala University, from the group of Prof. Ulf Landegren

## Goal

Enable tomorrow's precision medicine today

# The Significance

Current methodologies for mutation detection cannot reach high sensitivities required to capture low mutation burden




**Enabling Precision Medicine**

**Residual mutations**

**Relapse detection**

**Resistance markers**

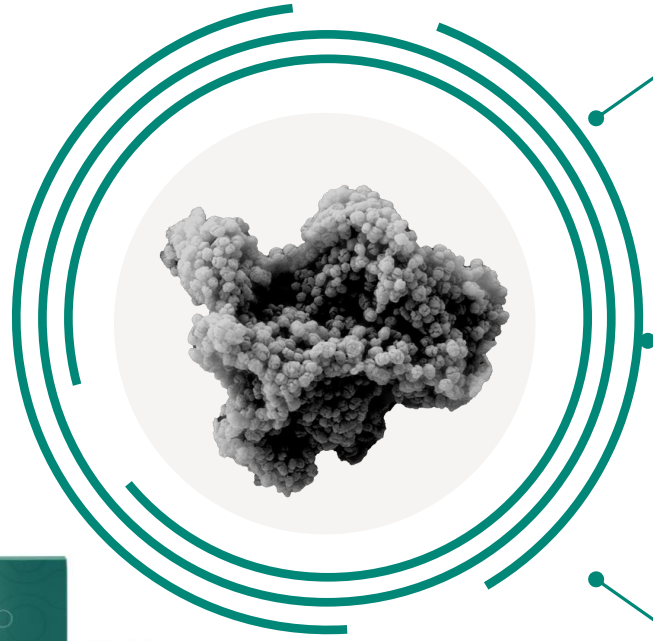
**Therapy response**

**Drug inclusion**

# Our superRCA Technology

Rarity™ superRCA converts DNA into fluorescent particles, enabling ultra-sensitive mutation detection using flow cytometry.

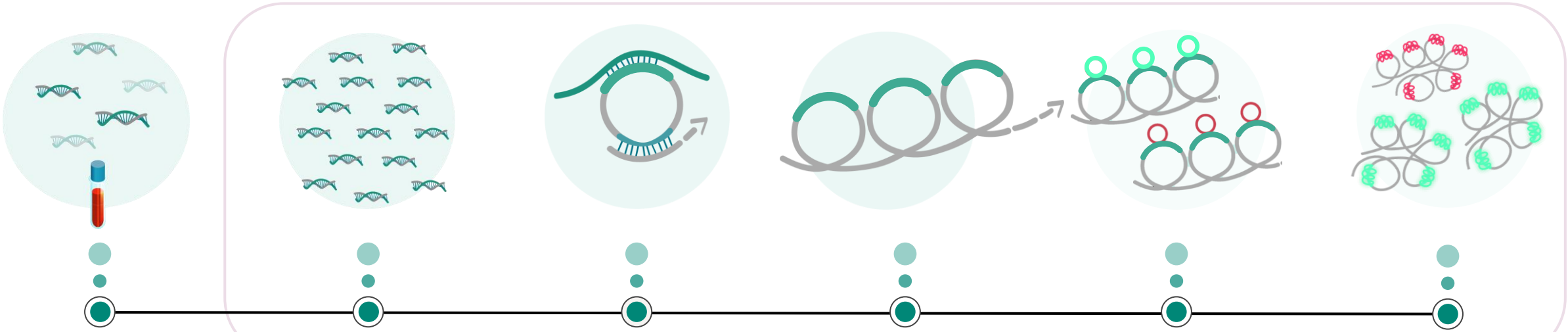
Detect 1 mutant in 100,000 events.



*The superRCA Accessory Kit and superRCA Mutation Kit*

- Up to 100 times more sensitive compared to current technologies
- Scalable solution, enabling decentralized testing
- Leverage standard equipment thus capital investments are not required

# superRCA Workflow



## Sample Extraction

Upstream of the superRCA assay, DNA is extracted from any liquid biopsy

## Amplification

The DNA undergoes a selective 10 step PCR amplification

## Ligation

Padlock probes ligate to the target DNA, circularizing the template DNA

## First RCA

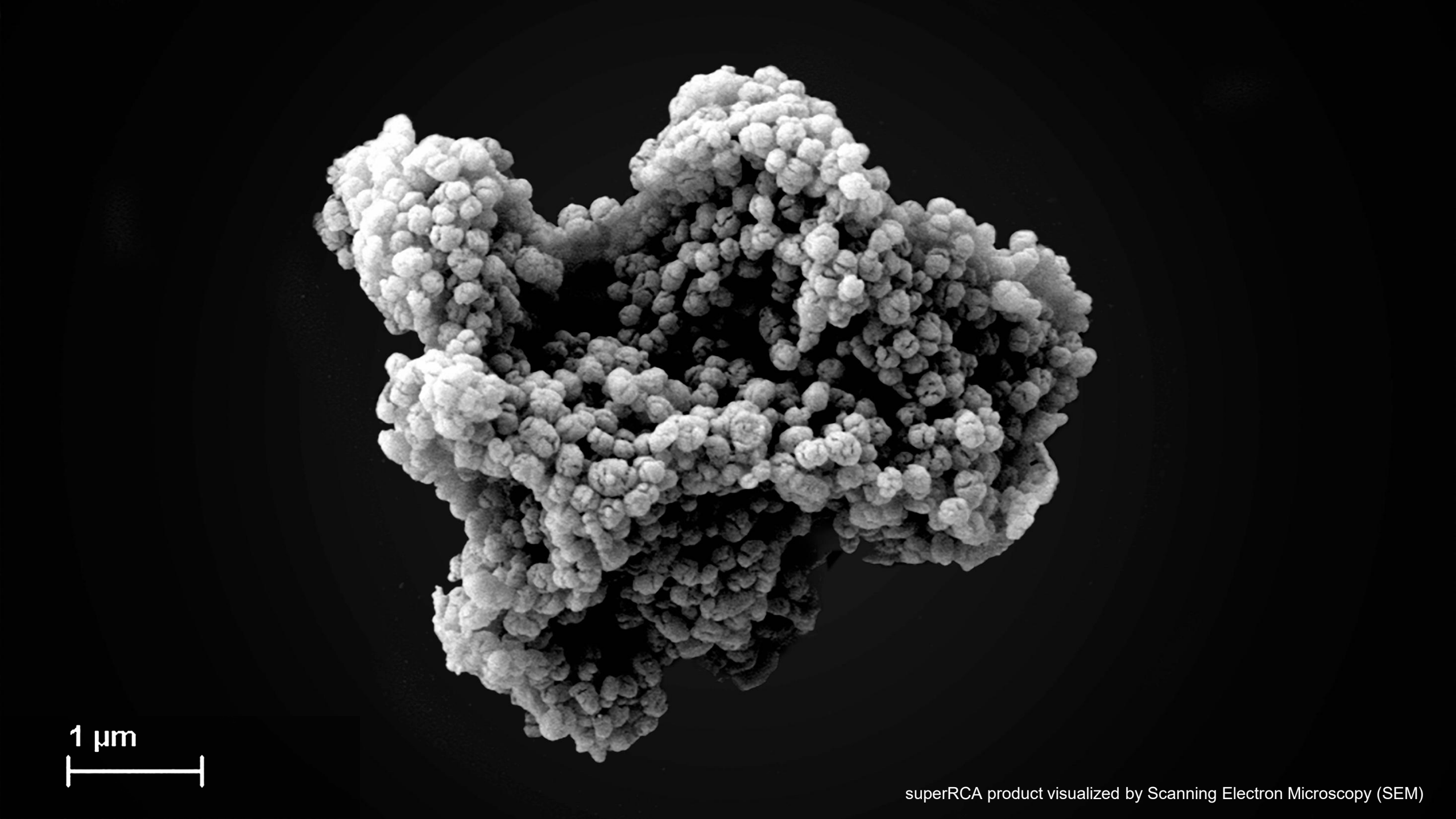
Rolling Circle Amplification (RCA) ensues, building a long, repeated ssDNA strand

## Genotyping

Genotyping probes specific to the wild type or mutant version of the sequence bind to the respective regions

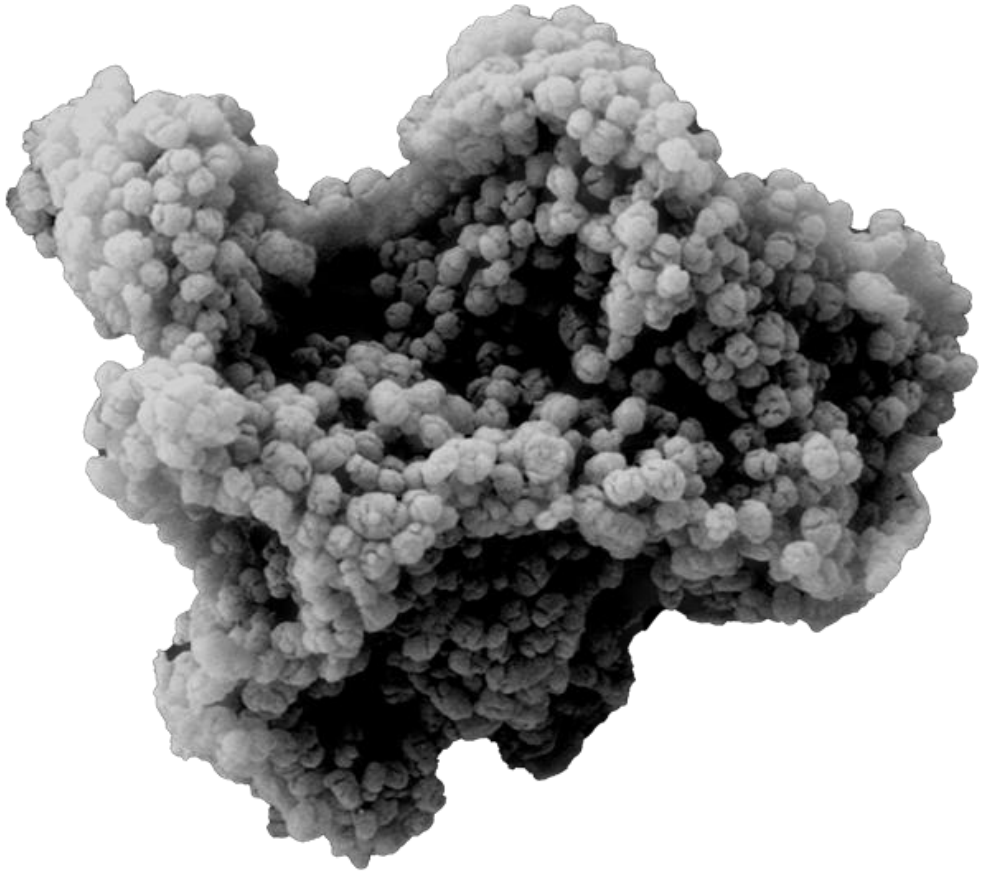
## superRCA

A second RCA step begins, further amplifying the genotyped sequences. Fluorescent tags are added that distinguish the wild type and mutant superRCA molecules

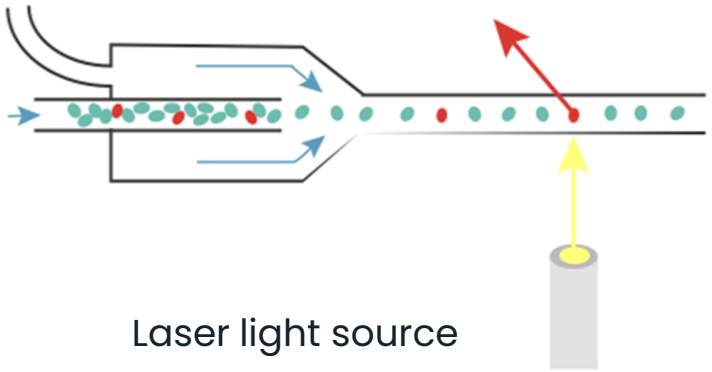



superRCA product visualized by Scanning Electron Microscopy (SEM)

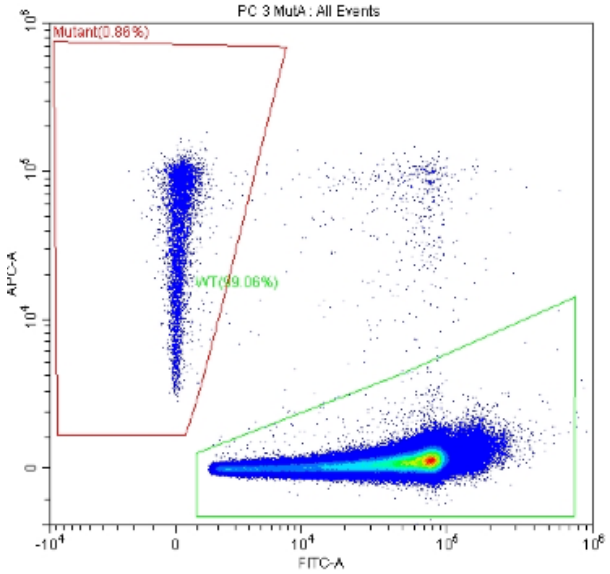
# superRCA Readout



Fluorescence emitted from superRCA products



Laser light source




**VAF% obtained**

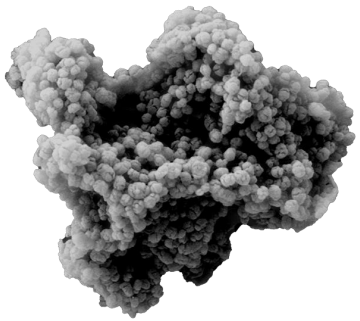
# What makes superRCA unique?

01

**High DNA input** permits detection of very low mutation frequencies

02

The high number of events generated - allows for **better precision**



03

The dual RCA process **transforms the DNA into a large molecule,** the size of a small cell

04

Leverages **standard flow cytometer** for read out

05

**Multi factor genotyping** minimizes false positives and reduces the background

06

**Simple process and interpretation** (no bioinformatics) enables any lab to adopt technology with limited training

# superRCA Workflows

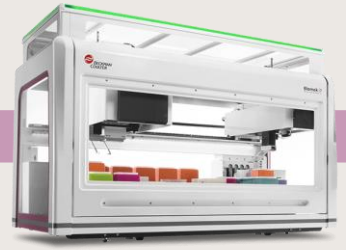
## Manual workflow

**Can be implemented quickly** as a manual workflow within 1-2 days

Time to get labs onboarded and up-and-running is short

**Read-out on all commercially available flow cytometers**

## Automated workflow



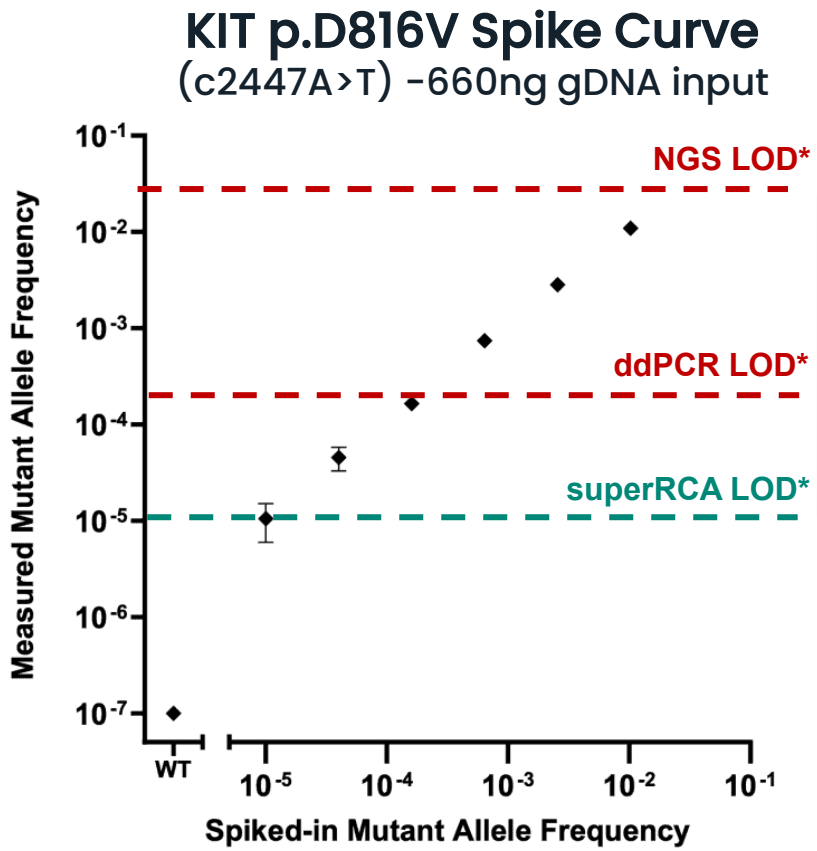
**Can easily be automated** on most lab automation platforms with an on-deck thermocycler

Enabling high throughput, reduced variation and increased quality

**Approximately 5.5h Assay Turn Around Time**
(Operated in 96 well format with 1.5h hands on time)

# Applications and Use Cases

# Pushing Boundaries in Sensitivity: *KIT* D816V

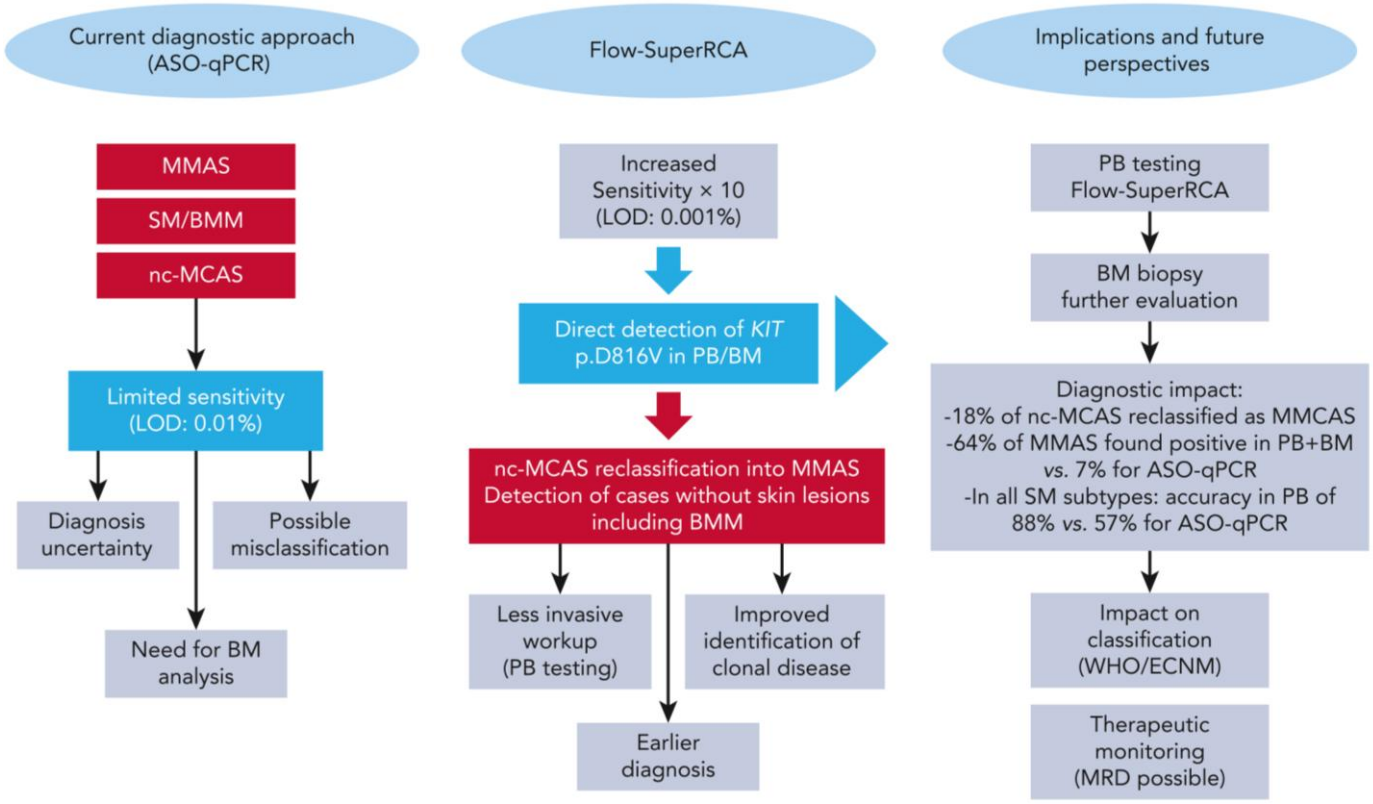


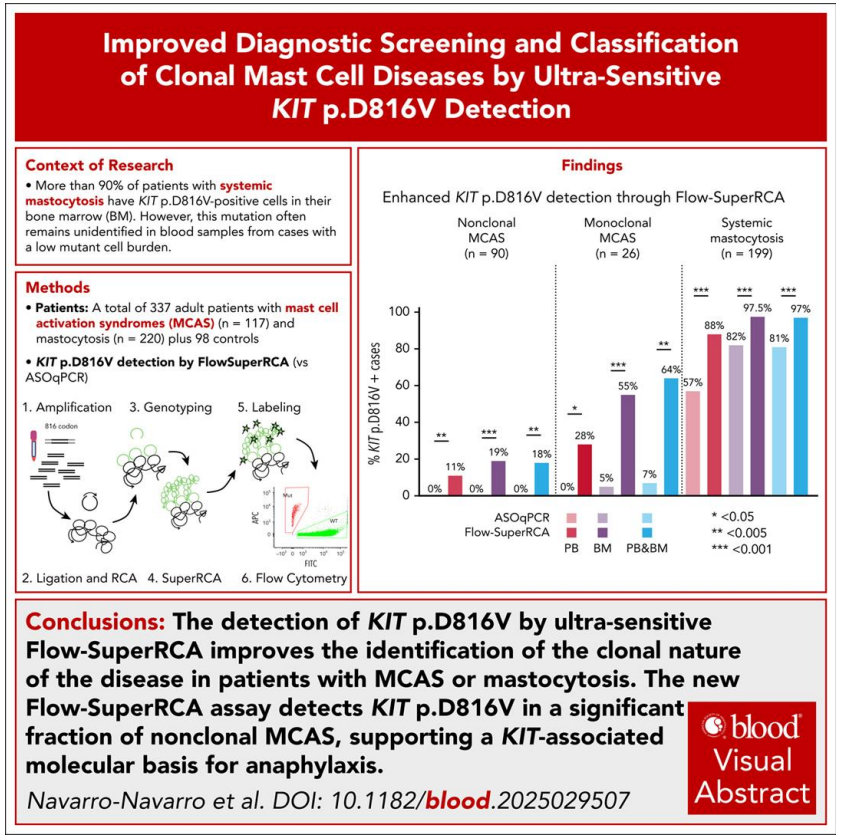

Range where mutations would not have been detected with other, less sensitive technologies.

Low background & high precision enables mutation detection capabilities beyond currently commercially available technologies

*Source: Ultra-sensitive KIT Testing Uncovers Previously Undetected KIT Mutations in Patients With ISM: Results From the PIONEER Trial*

# Case Study – *KIT* D816V



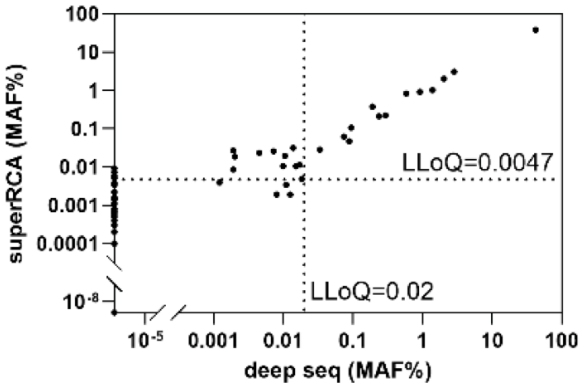



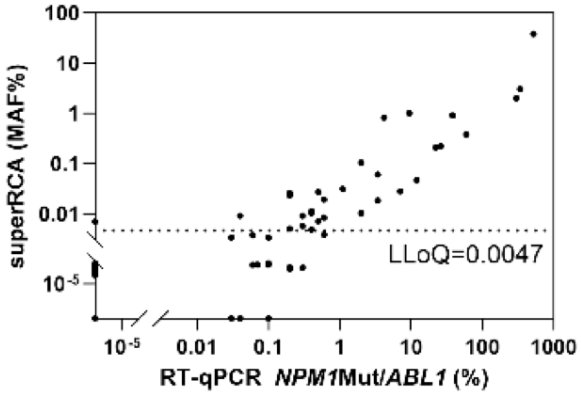

*Yannick Chantran, Michel Arock, MASTering KIT: enhanced sensitivity for refined diagnosis, Blood, 2025*

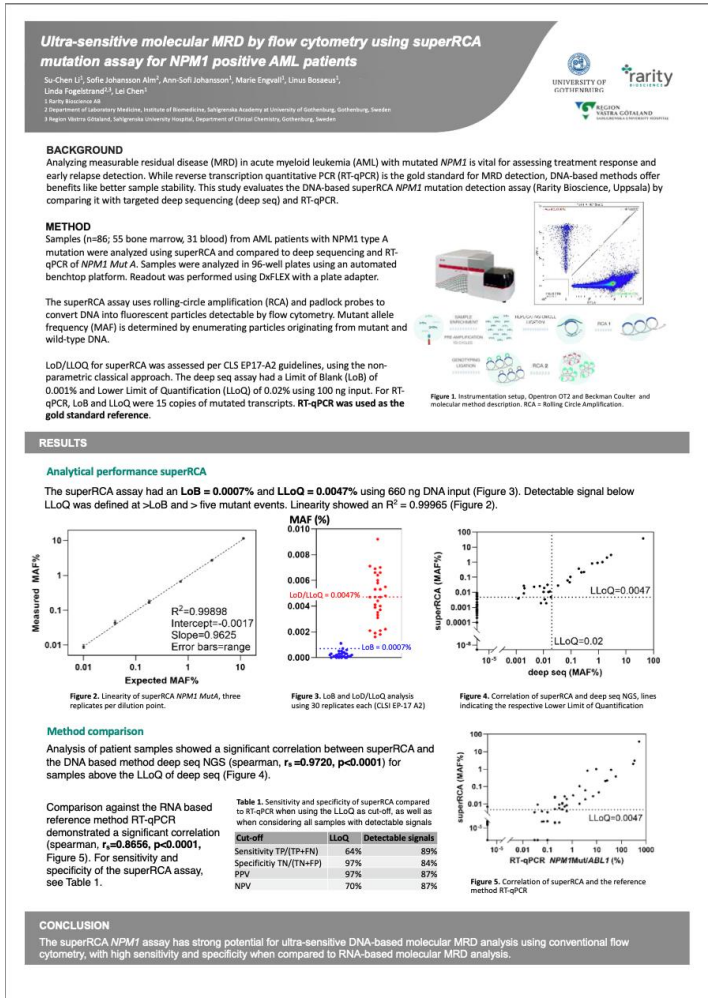
# Case Study – *NPM1*

## Key Points

The study evaluated the superRCA assay for detecting NPM1 mutations in AML patients and showcases how the superRCA assay offers higher sensitivity and specificity for MRD detection using flow cytometry, compared to RNA-based methods like RT-qPCR.



**Ultra-sensitive molecular MRD by flow cytometry using superRCA mutation assay for NPM1 positive AML patients**

Su-Chen Li[1], Sofie Johansson Alm[2], Ann-Sofi Johansson[1], Marie Engvall[1], Linus Boseaus[1], Linda Fogelstrand[2,3], Lei Chen[1]

1 Rarity Bioscience AB
2 Department of Laboratory Medicine, Institute of Biomedicine, Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden
3 Region Västra Götaland, Sahlgrenska University Hospital, Department of Clinical Chemistry, Gothenburg, Sweden

**BACKGROUND**

Analyzing measurable residual disease (MRD) in acute myeloid leukemia (AML) with mutated *NPM1* is vital for assessing treatment response and early relapse detection. While reverse transcription quantitative PCR (RT-qPCR) is the gold standard for MRD detection, DNA-based methods offer benefits like better sample stability. This study evaluates the DNA-based superRCA *NPM1* mutation detection assay (Rarity Bioscience, Uppsala) by comparing it with targeted deep sequencing (deep seq) and RT-qPCR.

**METHOD**

Samples (n=86; 55 bone marrow, 31 blood) from AML patients with NPM1 type A mutation were analyzed using superRCA and compared to deep sequencing and RT-qPCR of *NPM1 Mut A*. Samples were analyzed in 96-well plates using an automated benchtop platform. Readout was performed using DxFLEX with a plate adapter.

The superRCA assay uses rolling-circle amplification (RCA) and padlock probes to convert DNA into fluorescent particles detectable by flow cytometry. Mutant allele frequency (MAF) is determined by enumerating particles originating from mutant and wild-type DNA.

LoD/LLOQ for superRCA was assessed per CLS EP17-A2 guidelines, using the non-parametric classical approach. The deep seq assay had a Limit of Blank (LoB) of 0.001% and Lower Limit of Quantification (LLoQ) of 0.02% using 100 ng input. For RT-qPCR, LoB and LLoQ were 15 copies of mutated transcripts. **RT-qPCR was used as the gold standard reference**.

**Figure 1.** Instrumentation setup, Opentron OT2 and Beckman Coulter and molecular method description. RCA = Rolling Circle Amplification.

**RESULTS**

**Analytical performance superRCA**

The superRCA assay had an **LoB = 0.0007%** and **LLoQ = 0.0047%** using 660 ng DNA input (Figure 3). Detectable signal below LLoQ was defined at >LoB and > five mutant events. Linearity showed an $R^2$ = 0.99965 (Figure 2).

**Figure 2.** Linearity of superRCA *NPM1 MutA*, three replicates per dilution point.

**Figure 3.** LoB and LoD/LLoQ analysis using 30 replicates each (CLSI EP-17 A2)

**Figure 4.** Correlation of superRCA and deep seq NGS, lines indicating the respective Lower Limit of Quantification

**Method comparison**

Analysis of patient samples showed a significant correlation between superRCA and the DNA based method deep seq NGS (spearman, $r_s$=**0.9720, p<0.0001**) for samples above the LLoQ of deep seq (Figure 4).

Comparison against the RNA based reference method RT-qPCR demonstrated a significant correlation (spearman, $r_s$=**0.8656, p<0.0001,** Figure 5). For sensitivity and specificity of the superRCA assay, see Table 1.

**Table 1.** Sensitivity and specificity of superRCA compared to RT-qPCR when using the LLoQ as cut-off, as well as when considering all samples with detectable signals

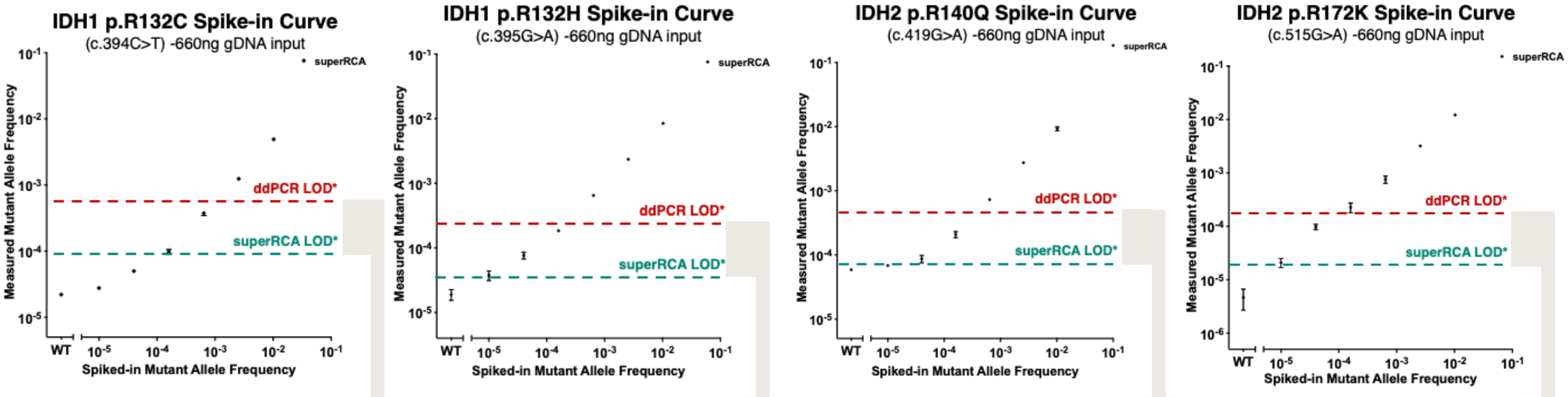
| Cut-off | LLoQ | Detectable signals |
|---|---|---|
| Sensitivity TP/(TP+FN) | 64% | 89% |
| Specificity TN/(TN+FP) | 97% | 84% |
| PPV | 97% | 87% |
| NPV | 70% | 87% |

**Figure 5.** Correlation of superRCA and the reference method RT-qPCR

**CONCLUSION**

The superRCA *NPM1* assay has strong potential for ultra-sensitive DNA-based molecular MRD analysis using conventional flow cytometry, with high sensitivity and specificity when compared to RNA-based molecular MRD analysis.

# Pushing Boundaries in Sensitivity: *IDH1/2*




Low background & high precision enables mutation detection capabilities beyond currently commercially available technologies

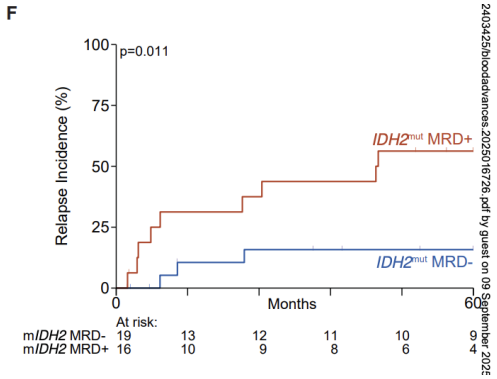
Range where mutations would not have been detected with other, less sensitive technologies.

# Case Study – *IDH1/IDH2*

## Key Points

Mutant IDH2 persistence in complete remission is associated with higher relapse risk in mutant IDH2 AML patients without concomitant NPM1 of FLT3-IDT mutations.

Thus, mutant IDH2 appears to be a potentially useful novel molecular MRD marker with prognostic significance in AML.




## superRCA and deepSeq correlation

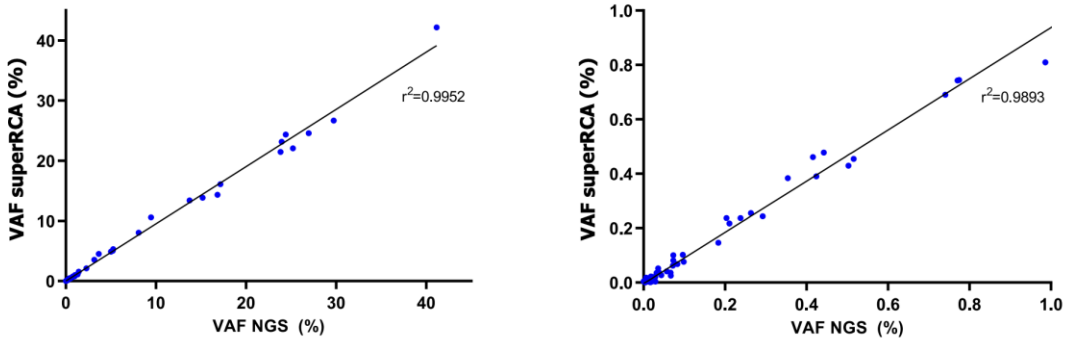



**Figure S3. Correlation of VAFs of persisting *IDH* mutations in CR as detected by NGS and superRCA**

A. Correlation of VAFs detected by superRCA and VAFs detected by NGS of all patients (n=128)8)
B. Correlation of low VAFs detected by superRCA and VAFs detected by NGS of all patients (VAF<1%)

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RESEARCH ARTICLE | JULY 17, 2025

**Utility of IDH1/2 mutations as biomarkers for detection of measurable residual disease in acute myeloid leukemia**

Emma L. Boertjes, Christian M Vonk, François G. Kavelaars, Marie Engvall, Sofia Nordin, Lisanne Beugelink, Melissa Rijken, Jolinda Konijnenburg, Jurjen Versluis, Bob Löwenberg, Peter J.M. Valk

Check for updates

*Blood Adv* bloodadvances.2025016726.
https://doi.org/10.1182/bloodadvances.2025016726 Article history

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### Key Points

- Persistence of IDH1 mutations in CR is not associated with increased risk of relapse in AML.
- Mutant IDH2 persistance in CR is associated with higher relapse risk in mutant IDH2 AML patients without concomitant mutant NPM1 or FLT3-ITD

Molecular measurable residual disease (MRD) assessment in acute myeloid leukemia (AML) patients has been established for only a few specific markers, i.e. mutant NPM1 and FLT3-ITD. Mutations in IDH1/2 are present in approximately 20% of AML patients. However, validation of mutant IDH1/2 MRD has been hampered by cohort size as well as the availability of highly sensitive and specific MRD detection assays. Here, we comprehensively investigate the impact of persisting IDH1/2 mutations in complete remission (CR) after intensive chemotherapy in a cohort of 163 newly diagnosed IDH-mutant AML patients enrolled in HOVON-SAKK clinical trials using a next-generation sequencing (NGS)-based approach, targeting all hotspot mutations in IDH1 (R132) and IDH2 (R140, R172). The high sensitivity (10-4) as well as the levels of persisting IDH1/2 mutations detected by the NGS-based approach were confirmed by an independent rolling circle amplification (superRCA) assay. We demonstrate that relapse risk was significantly increased in AML patients with measurable persisting IDH2 mutations (p=0.027, SHR:2.34), but not in patients with persisting mutant IDH1 (p=0.591, SHR:0.80). Moreover, the association of persistence of mutant IDH2 and increased risk of relapse was most pronounced in mutant IDH2 AML patients without concomitant NPM1 mutations or FLT3-ITD (p=0.011, SHR:5.29). Thus, mutant IDH2 appears a potentially useful novel molecular MRD marker with prognostic significance in AML.

# Summary and conclusions

- superRCA is a novel chemistry that **enables ultra-sensitive mutation detection**
- Improved tools could support clinicians in **uncovering previously undetected mutations in patients**
- The technology is highly scalable, **leveraging the existing install base of flow cytometry instruments**
- **The assays are easy to implement, available both** manual and automated. Easy interpretation - no bioinformatics

## For more reading about the technology

Article in Nature Communications
Ultra-sensitive monitoring of leukemia patients using superRCA mutation detection assays
Article in Cancers
Sensitive and Specific Analyses of Colorectal Cancer Recurrence through Multiplex superRCA Mutation Detection in Blood Plasma


We are enabling tomorrow’s precision medicine today

rarity bioscience.com